

### **AMENDMENTS TO THE CLAIMS**

This listing of claims will replace all prior versions, and listings, of claims in the application:

#### **Listing of Claims:**

1. (Currently amended) A method for a high sensitivity multiple microorganism detection which is a method for detecting two or more microorganisms in foods having different properties by culturing the microorganisms in a medium with glucose concentration of 0.15% or less, and/or in a medium with concentration of phosphate-buffer solution of 50 mM or more or in a medium with a buffer ability similar as that with concentration of phosphate-buffer solution of 50 mM or more, amplifying a plurality of target genes with a single PCR reaction tube and analyzing the same, comprising the following steps:  
(A) a step for extracting DNA of the target microorganisms to be detected, by treating at least with a lytic enzyme and/or bacteriocin having lytic activity, a surfactant and a protein denaturant; and  
(B) a step for performing Multiplex PCR by mixing a primer specific to the target microorganisms to be detected,  
wherein, at least one of the two or more microorganisms with different properties is *Listeria monocytogenes*.
2. (Original) The method of multiple microorganism detection according to claim 1, wherein a step to culture microorganisms under a culture condition where 1 CFU/100 g microorganisms become  $10^3$  CFU/ml or more after 24 h of culture, is included prior to the step of extracting DNA of the target microorganisms to be detected.
3. (Cancelled)
4. (Original) The method of multiple microorganism detection according to claim 3, wherein the specific primer is a primer consisting of base sequences shown by SEQ ID Nos: 5 and 6.

5. (Withdrawn) The method of multiple microorganism detection according to claim 1 or 2, wherein the two or more microorganisms with different properties comprise pathogenic *Escherichia coli* O157.
6. (Withdrawn) The method of multiple microorganism detection according to claim 5, wherein the specific primer is a primer consisting of base sequences shown by SEQ ID Nos: 1 and 2, or SEQ ID Nos: 7 and 8.
7. (Withdrawn) The method of multiple microorganism detection according to claim 1 or 2, wherein the two or more microorganisms with different properties comprise *Salmonella* spp.
8. (Withdrawn) The method of multiple microorganism detection according to claim 7, wherein the specific primer is a primer consisting of base sequences shown by SEQ ID Nos: 3 and 4, or SEQ ID Nos: 9 and 10.
9. (Previously presented) The method of multiple microorganism detection according to claim 1 or 2, wherein the microorganisms are cultured in a culture condition where the pH after culture becomes 5.1 or more.
10. (Cancelled)
11. (Previously presented) The method of multiple microorganism detection according to claim 1 or 2, wherein the extraction is performed after treating with a lytic enzyme and/or bacteriocin having a lytic activity, further treating with a surfactant and a protein denaturant, removing insoluble fractions by centrifugation, and by depositing DNA by alcohol precipitation.
12. (Previously presented) The method of multiple microorganism detection according to claim 1 or 2, wherein the lytic enzyme is Achromopeptidase and/or lysozyme.
13. (Previously presented) The method of multiple microorganism detection according to claim 1 or 2, wherein bacteriocin having lytic activity is Enterolysine.

14. (Previously presented) The method of multiple microorganism detection according to claim 1 or 2, wherein the surfactant is ethyleneoxide condensate of sorbitan monolaurate.
15. (Previously presented) The method of multiple microorganism detection according to claim 1 or 2, wherein the protein denaturant is Guanidine isothiocyanate.
16. (Previously presented) The method of multiple microorganism detection according to claim 1 or 2, wherein Multiplex PCR is performed by combining DNA consisting of base sequences shown by SEQ ID NOs: 1 to 6 at a total concentration of 750 nM or less as a primer.
17. (Withdrawn) The method of multiple microorganism detection according to claim 1 or 2, wherein Multiplex PCR is performed by combining DNA consisting of base sequences shown by SEQ ID NOs: 5 to 10 at a total concentration of 750 nM or less as a primer.
18. (Previously presented) The method of multiple microorganism detection according to claim 1 or 2, wherein the food is edible meat or processed meat product.